

# A Review of Approaches to the Detection of Genetic Damage in the Human Fetus

by Richard B. Everson\*

Studies in experimental animals suggest links between genetic damage to the fetus and the etiology of several disorders, including fetal loss, teratogenesis, and cancer. Methods for measuring genetic damage directly in the human fetus could provide epidemiologists and clinical researchers with powerful tools for investigating similar associations in humans. Current methods potentially available for such studies include assays for mutagenic substances in human body fluids and for measuring modifications to genetic material at the three levels of organization of genetic material: the chromosome, the gene or specific locus, and chemical DNA. Results of studies using fetal tissues to investigate each of these end points are reviewed, emphasizing studies of chemical modifications to DNA nucleotides detected in the human placenta.

## Introduction

Damage to fetal genetic material could result in a number of immediate and long-term effects, including fetal loss, teratogenesis, germ or somatic cell mutation, or cancer. Interest in such postnatal effects of prenatal exposures was greatly intensified when epidemiologic studies linked prenatal exposure to diethylstilbestrol with vaginal cancer in adults, demonstrating that chemical agents can act as transplacental carcinogens in humans (1-3). There are, however, few observations indicating that transplacental carcinogenesis occurs in response to other chemical exposures in the clinical or epidemiologic literature. Probably the best additional examples of transplacental induction of cancer by a chemical are case studies showing that exposure to diphenylhydantoin induces neuroblastoma, but the number of affected children is small (4). More recent epidemiologic studies have also suggested associations between maternal exposure to *N*-nitroso compounds and childhood brain tumors (5) and hydrocarbon-related occupations and childhood leukemia (6), but these studies require confirmation.

In contrast to the rarity of epidemiologic models for human transplacental carcinogenesis, there are many examples of transplacental carcinogenesis in experimental animals (4,7-10). Table 1 reviews several principles extracted from this literature. These experimental studies have shown that exposure of fetal tissues to factors capable of causing cancer may be common, and the studies raise the question: does the clinical and epidemiologic literature lack other examples because transplacental carcinogenesis is not occurring in humans

Table 1. Principles of transplacental carcinogenesis derived from animal studies.

---

Prenatal exposure to over 40 chemicals, including most classes of chemical carcinogens active in postnatal life, can induce tumors in experimental animals.
Carcinogens (initiators) administered transplacentally can increase sensitivity to later carcinogenic (initiating or promoting) exposures.
Many tumors resulting from transplacental exposures occur in adulthood and at the same sites as spontaneously occurring tumors.
Compounds present in tobacco smoke and cigarette smoke condensate are active transplacental carcinogens in animals.
The fetus has greater sensitivity to some carcinogens than the adult, so that estimates of doses necessary for transplacental carcinogenesis cannot be accurately predicted from effects in adults.

---

or because we do not have the tools to detect transplacental carcinogenesis in humans?

A search for possible exposures that could be used as models for investigating human transplacental carcinogenesis led to consideration of cigarette smoking. Maternal smoking is associated with increased rates for spontaneous abortion, perinatal mortality, congenital defects, and low birth weight. Maternal smoking also causes large increases in some forms of human placental metabolism (11). These data indicated that transplacental exposure to constituents of cigarette smoke occurs and has biologically important effects. Animal studies showed that several constituents of cigarette smoke and cigarette smoke condensate itself are transplacental carcinogens. Taken together, these findings suggested the possibility that individuals transplacentally exposed to maternal smoking may be at increased cancer risk in adult life (4). Investigations of this possibility offer an opportunity both to study this specific hypothesis and

\*Epidemiology Branch, National Institute of Environmental Health Sciences, P. O. Box 12233, Research Triangle Park, NC 27709.

to use these investigations to develop approaches for similar studies of other exposures.

Using a traditional epidemiologic approach in which information gathered by questionnaires is linked with health outcomes from medical records, studies investigating the effects of gestational exposure to maternal smoking were proposed (4) and then piloted (12,13). In these studies, evaluation of effects of transplacental exposures was complicated by significant associations between cancer risk and passive exposure to smoking by the mother or father. They were also hampered by relatively low frequencies of maternal use of cigarettes in the area in which the studies were conducted. Overall, in the United States, large numbers of women began smoking during the 1920s, so that transplacentally exposed individuals are only now reaching the age at which a high frequency of cancer would be expected. Despite these difficulties, a significant association between maternal smoking and elevated risk of hematopoietic malignancies was observed [relative risk (*RR*) = 2.7]. Although numbers were small and not all of the risks were significant, diseases with elevated risk included Hodgkin's disease (*RR* = 4.4), non-Hodgkin's lymphomas (*RR* = 1.7), and the acute leukemias (*RR* = 8.8) (12). A methodological study investigating the consistency between parental smoking histories obtained independently from the cancer victims and the victims' parents was also completed; agreement was good qualitatively, but relatively poor quantitatively (14). Links between maternal smoking and cancer risk are being explored further in case-control studies and will not be discussed further here.

In part, consideration of the limitations of using traditional epidemiologic methods to investigate transplacental carcinogenesis prompted consideration of alternative strategies that integrate laboratory and epidemiologic studies. Rather than attempting to establish empirically direct links between exposure and disease, such biochemical or molecular approaches to environmental epidemiology investigate discrete stages in the pathophysiological sequence of events intervening between exposure and effect, including measures of external exposures, internal exposures, biologically effective exposures, and biological responses (15). This approach could be further described as the use of laboratory tests to define exposures, susceptibility factors, disease outcomes, and the pathophysiological sequence of events linking exposure and disease in epidemiologic studies of (noncardiovascular) chronic diseases.

Carcinogenesis studies in experimental animals indicate that an early step in the effects of many chemical carcinogens is damage to genetic material. Likewise, most chemical agents that caused transplacental carcinogenesis in experimental animals were of chemical classes that are likely to damage DNA. Following these leads, it seems most appropriate to review methods that have been used to detect genetic damage directly in human fetal tissues. The specific approaches that will be included here are summarized in Table 2. Animal

**Table 2. Detection of mutagens in body fluids by bacterial assays.**

Site	Genetic damage
Chromosome	Aberrations, micronuclei, sister chromatid exchanges
Gene or specific locus	Lymphocytes resistant to 6-thioguanine
Chemical	DNA adducts

studies using several of these end points to detect fetal damage were recently reviewed by Henderson (16).

## Approaches to Measuring Exposure to Mutagens and Genetic Damage in the Fetus at Three Levels of Organization of Genetic Material

### Bacterial Mutagenesis Assays of Amniotic Fluid

Bacterial mutagenesis assays of human body fluids provide a method for detecting levels of internal exposure to mutagens. Mutagens observed may be of environmental or endogenous origin. These assays are conducted by obtaining specimens of body fluids, either sterilizing the samples or extracting them (typically by means of miniature columns of resins), and testing them in bacterial mutagenesis assays. Most studies have used a protocol modified from that described by Yamasaki and Ames (17), in which specimens were extracted with XAD-2 resins and tested with the Salmonella/mammalian microsomal plate assay. Further description of the methods, results for specimens of human urines, and the advantages and limitations of this approach can be found in two reviews (18,19).

Three studies have applied bacterial mutagenesis assays to specimens of human amniotic fluid (20–22). One of these studies examined only specimens taken at diagnostic amniocenteses obtained early in the second trimester (20). The small volume available (less than 10 mL) limited the sensitivity of these assays. While assays of similar volumes of urine from smokers were more mutagenic than urine specimens from nonsmokers, comparison of amniotic fluid specimens from 94 nonsmokers and 28 smokers showed no association between maternal smoking and mutagenicity of amniotic fluids. Regardless of the smoking status of donors, however, specimens of amniotic fluid showed small increases in mutagenicity compared with concurrent solvent controls. These increases suggest the presence of small amounts of mutagenic activity in many of the amniotic fluid specimens not associated with maternal smoking.

A subsequent study also found no increases in mutagenicity among amniotic fluid specimens obtained during the second trimester (21). However, concentrates of larger volumes of amniotic fluid obtained at term from some of the heavy smokers studied had increased mutagenic activity compared with similar specimens from

nonsmokers (21,22). While these studies demonstrate that exposure to mutagens in fetal tissues is occurring in heavy smokers, they do not indicate whether exposure starts only after several weeks of gestation or only among heavy smokers. Such questions deserve further study using more sensitive assays, such as the method described by Kado (23) or other techniques. More sensitive techniques may also help define the origin of mutagenicity not related to smoking.

## Chromosomal

Three tests have been used to detect chromosomal damage to genetic material in the fetus: assays for chromosomal aberrations, micronuclei, and sister chromatid exchanges in cord blood lymphocytes. Although not all studies are consistent, the majority of studies have found that in adults, smokers have increased frequencies of chromosomal aberrations (24) and sister chromatid exchanges (24-26). Studies of the effect of smoking on frequencies of micronuclei in lymphocytes have given conflicting results (24,25). One study reported small increases in frequencies of SCEs induced by mitomycin C among passive smokers (27).

Although karyotypes are often obtained from infants for diagnosis of genetic diseases, few studies have investigated somatic cell damage by analyses of such specimens for chromosomal aberrations. Two brief reports found no evidence of increased chromosomal aberrations among cord blood lymphocytes of smokers (24,28). These studies analyzed cord blood specimens from the offspring of a total of 56 smokers and 42 nonsmokers. In addition, a study of low birth weight infants found no association between birth weight and frequencies of chromosomal aberrations, but did not study the independent effect of smoking (29). Smoking was also not considered in a study that measured the frequency of micronuclei in 28 cord blood specimens (30).

Data from four studies (31-34) evaluating the frequencies of sister chromatid exchanges among women who smoked during pregnancy and cord blood specimens from their offspring are presented in Figure 1. Each of these studies found appreciably lower rates of sister chromatid exchanges in cord blood lymphocytes than in maternal blood lymphocytes. In two studies (33,34) smoking was associated with increased SCE values in maternal blood. Lack of a smoking effect on maternal cells in the other studies may have been due to the small numbers of subjects studied, concentration of 5-bromo-2-deoxyuridine used, the relatively low numbers of cigarettes smoked or relatively brief duration of smoking for most women studied (33,34). None of the studies, including that by Lundgren et al. (34), which showed a clear smoking effect in maternal cells and used several assay conditions designed to enhance smoking effects (Fig. 1), found a statistically significant association between smoking and SCE frequencies in cord blood. A total of 134 subjects were included in these studies, with subjects about equally divided between smokers and nonsmokers. Unlike chromosomal aber-

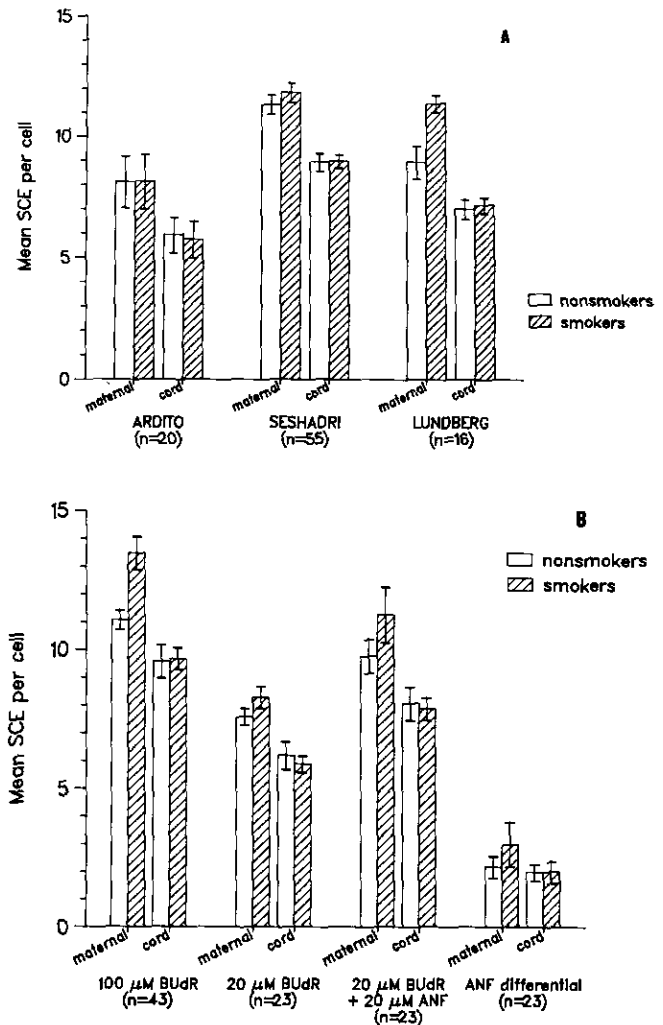


FIGURE 1. Frequencies of sister chromatid exchanges in maternal and cord blood, by smoking status of the mother during pregnancy. (A) Three studies using low levels of 5-bromo-2-deoxyuridine (BUdR) in the culture media. Data are from (31-33). Frequencies of sister chromatid exchanges were consistently higher in maternal than in fetal cells. The only significant difference between smokers and nonsmokers were higher levels in maternal cells in the study by Lundberg et al. (33). (B) A study in which SCE levels were compared for assays done with different levels of BUdR in the culture media and with and without  $\alpha$ -naphthoflavone, which previous studies suggested would enhance the frequency of SCEs among smokers. Data are from (34). Smoking was associated with significantly higher frequencies of SCEs only for maternal cells using high levels (100  $\mu$ M) of BUdR in the culture media. Bars indicate one SE.

rations, whose low frequency provides relatively little statistical power, the aggregate statistical power of the SCE studies should have been substantial. Taken together, these measurements eliminate more than a small effect of smoking on SCEs in cord blood.

Two studies (32,35) showed no differences in SCE frequencies between healthy nonpregnant and pregnant women, indicating that pregnancy itself does not affect SCE values. A third study (36) did report higher SCE and aberration rates among pregnant women in India

(perhaps, the authors note, because of folate deficiency during pregnancy). In other data from studies of SCEs in cord blood, Seshadri et al. (32) reported an association between moderate or high consumption of alcohol by the mother and high frequencies of SCEs in maternal blood but not cord blood samples. In addition to these studies, Hatcher and Hook (37) found no association between the SCE frequencies in cord blood specimens and either rates of chromosomal aberrations or birth weight. Both their data and data of Das et al. (38) suggest rapid reductions in SCE frequencies after birth. Neither of these latter two studies examined effects of smoking on SCE frequencies.

### Gene or Specific Locus Effects

Although there currently is no well-established method for detecting genetic damage occurring *in vivo* at the level of the gene or specific locus in human somatic cells, two laboratory approaches are in advanced stages of development. One of these assays, developed by Strauss and Albertini (39), analyzes peripheral blood lymphocytes for resistance to 6-thioguanine. Resistant cells have lost activity of the enzyme hypoxanthine-guanine phosphoribosyl transferase (HGPRT), presumably due to mutational loss of gene function, making them unable to activate the 6-thioguanine to metabolites that interfere with lymphocyte activation or proliferation. In the initial versions of this assay, resistant cells are scored autoradiographically by their ability to take up tritiated thymidine (39). Recent technical developments provide an alternative means for scoring: the ability of lymphocytes to make clones (40-43). Genetic material from clones obtained by the latter approach can then be analyzed further, allowing demonstration of the molecular basis of changes at the HGPRT locus. Using this approach, in early studies, restriction-fragment analysis using Southern blots showed substantial gene alterations not visible cytogenetically in about 50% of 6-thioguanine-resistant lymphocyte clones. Changes included apparent deletions, new bands, and exon amplifications (44,45).

A second technique for direct measurement of putative mutations in somatic cells is the detection of gene loss by quantitating erythrocytes that have lost expression of glycophorin-A membrane antigens. Individuals heterozygous at the glycophorin locus should have two glycophorin antigens on the surface of their red cells. This assay quantitates the frequency at which red cells from heterozygous individuals do not react with monoclonal antibodies against one of the antigens. Advantages of the glycophorin-A assay are that it requires fewer *in vitro* manipulations than the lymphocyte assays, it uses a more homogeneous target tissue, and it has a more automated scoring system (46,47).

At this time only the assay for 6-thioguanine has been used to study specimens of cord blood. Albertini et al. (48) studied specimens from 58 normal pregnancies using the autoradiographic assay. The mean value for variant cells was 2 per  $10^6$  cells. Logistic regression analysis

suggested that an elevated frequency of variant cells was associated with use of caffeine ( $p < 0.03$ ); too few smokers were studied to allow investigation of smoking effects. Henderson et al. (43) recently reported results from nine cord blood specimens assayed using the cloning technique. Variant frequencies were markedly lower in these cord blood specimens than in adult blood specimens, but the sample was too small to allow analysis of determinants of variant frequencies from either specimen source.

### Chemical Modifications of DNA (DNA Adducts)

Chemical alteration of DNA is a fundamental mechanism by which environmental agents cause adverse effects in genetic material. Techniques used to measure chemical modifications to DNA include assays for unscheduled DNA synthesis, DNA strand breaks, and chemical addition products (adducts). While each of these approaches have been able to detect changes following relatively high doses of exposure in experimental studies, assays with sufficient sensitivity to measure *in vivo* effects in human subjects have become available only recently. The assays with the greatest sensitivity are procedures for measuring DNA adducts.

In experimental studies, the incorporation of radio-labeled chemicals into DNA and direct physiochemical analysis for adducts (e.g., by use of high-performance liquid chromatographic separation and fluorescent detection of adducts) are the analytical approaches most frequently used to measure DNA adducts. The former approach would require administration of high levels of radioactivity, and the latter approach has not been sufficiently sensitive to measure formation of adducts *in vivo* in humans.

Because of limitations in the techniques commonly used to detect DNA adducts, studies of DNA adducts formed *in vivo* in human tissues used two other recently developed methods for detecting adducts. One method, an immunologic approach, measures adducts in DNA from tissues using radioimmunoassays or enzyme-linked immunosorbant assays (ELISAs) conducted with antibodies against DNA adducted with known chemicals (49-52). The second method,  $^{32}\text{P}$ -postlabeling (53,54), is performed by isolating DNA, digesting it to mononucleotides, and postlabeling the mononucleotide with radioactive phosphate using an enzymatic process that is highly specific for DNA nucleotides, including adducts. Nucleotides containing aromatic adducts are then separated from normal nucleotides by thin-layer chromatography, and autoradiograms are made showing maps, or fingerprints, of DNA adducts. Levels of the adducts can then be estimated by the density of the autoradiograms or, more accurately, by scraping the thin-layer chromatograms and quantitating the radioactivity present (53,54).

$^{32}\text{P}$ -Postlabeling was used recently to demonstrate DNA adducts in fetal mouse tissues after the administration of chemical carcinogens to pregnant mice (55).

<sup>32</sup>P-Postlabeling also demonstrated that chronic administration of diethylstilbestrol results in the formation of DNA adducts in the kidney of male Syrian hamsters (56). Adduct formation was specific for the kidney, which is also the specific target organ for diethylstilbestrol carcinogenesis in this species. Subsequent study demonstrated that the adducts were a result of estrogen induction of endogenous chemicals to bind to DNA rather than adducts directly formed by estrogen (57).

To investigate whether modifications to fetal DNA could be detected in humans by these techniques, 43 specimens of human placental tissue from smokers and nonsmokers were analyzed by both of these approaches. Methods and results of this study are described in more detail elsewhere (52,58). Briefly, women were enrolled at about the seventh month of pregnancy, their medical records were abstracted and questionnaires were administered, blood specimens were obtained at the time of enrollment into the study, and maternal blood, cord blood, and placental specimens were gathered at the time of parturition. In addition to demographic data, the questionnaires obtained detailed histories of the use of cigarettes and exposures to potentially toxic chemicals during pregnancy. Blood specimens were analyzed for three biochemical markers of exposure to tobacco smoke: carboxyhemoglobin, cotinine (the major metabolite of nicotine), and thiocyanate. Placental specimens were analyzed for the presence of DNA adducts using antibodies to DNA modified with benzo[a]pyrene dihydrodiol epoxide (BPDE-I) in ELISA assays and with the adduct intensification modification (54) of the <sup>32</sup>P-postlabeling assay (52,58,59).

Although results of the ELISA assays indicated that placental DNA contained material reacting with antibodies against BPDE-I modified DNA, levels were similar for specimens from nonsmokers and smokers (52). The <sup>32</sup>P-postlabeling assay detected a total of seven chromatographically distinct adducts. The adduct observed most commonly, adduct 1, was strongly associated with maternal smoking: it was observed in 22 of 24 specimens of smokers, but only in 3 of 19 specimens from nonsmokers. Levels differed dramatically between nonsmokers and smokers (Fig. 2). The other six adducts were found less frequently than adduct 1. Two of the six were present only in nonsmokers; two other adducts were found almost exclusively in smokers. The small numbers of study subjects made it difficult to determine whether the latter adducts are actually associated with smoking (52,58). Recent unpublished data from a larger group of study subjects allowed more quantitative analysis of associations between adduct levels and the intensity of exposure to smoking as determined by questionnaire and biochemical data (carboxyhemoglobin, cotinine, and thiocyanate levels), effects of confounding factors on adduct levels, and associations between adduct levels and birth weight [which is known to be 200 to 300 g less among the offspring of smokers (60,61)]. Estimates of intensity of exposure to tobacco smoke from questionnaire data had much weaker associations with adduct levels than estimates from the biochemical

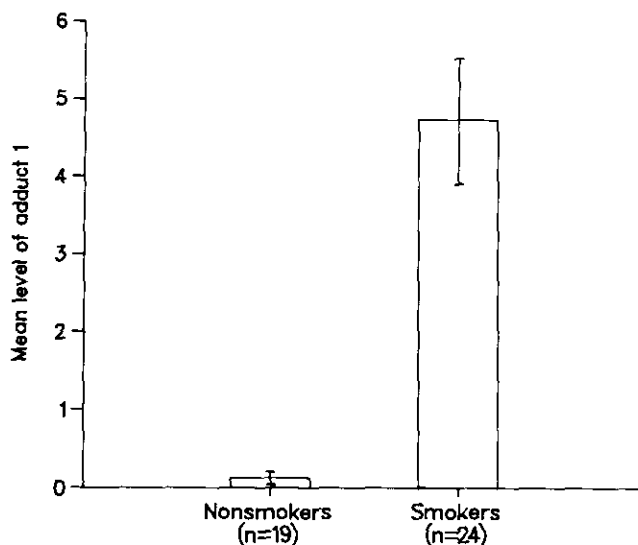


FIGURE 2. Mean levels of adduct 1 estimated from the <sup>32</sup>P-postlabeling assay, by smoking status of the mother during pregnancy. Bars indicate one SE. Data are from (58).

data, so that quantitative relationships might not have been recognized if only questionnaire data were available. Among smokers, levels of adducts were better predictors of decreased birth weight and infant length at birth than biochemical or questionnaire data (62).

Although many research questions can be addressed without knowing the chemical structure of the adducts, the identity of chemicals causing the adducts and their structure is of great interest. Levels of adducts detected with the very sensitive <sup>32</sup>P-postlabeling procedure are very low; however, the best estimate for the highest levels of adduct 1 in placentae is about one adduct in  $2 \times 10^8$  nucleotides (63). Thus, about 100 kg of placenta would have to be processed to recover 1  $\mu$ g of adduct, making it difficult to isolate sufficient quantities of adduct for identification by standard analytical chemical methods. Other approaches are therefore being used to identify adduct 1. One approach is cochromatography of adducts found in placental specimens with adducts from genotoxic aromatic chemicals known to be in tobacco smoke. These studies indicated that adduct 1 was not formed by benzo[a]pyrene, benz[a]anthracene, dibenz[a,h]anthracene, pyrene, chrysene, flouranthene, benzo[g,h,i]perylene, 4-aminobiphenyl,  $\beta$ -naphthylamine, or the isomeric methyl or ethyl derivatives of aniline (52). While similar studies with other chemicals continue, another approach is under investigation. This approach relies on the observation that cigarette smoke condensate painted on mouse skin give adducts that cochromatograph with adduct 1 under multiple chromatographic conditions (63). By chemically subfractionating the cigarette smoke condensate and following fractions containing adduct 1, it should eventually be possible to identify this and other adducts. These two approaches may serve as models for the identification of chemical modifications to DNA in tissues from individuals with environmental or occupational exposures.

## Discussion

### Strengths and Limitations of Studies on the Effects of Smoking

The comprehensiveness and statistical power of studies reviewed here vary widely. Only preliminary data are available for several approaches to measurement of genetic damage, including studies of chromosomal aberrations, micronuclei, and 6-thioguanine resistant lymphocytes. Investigations of the presence of mutagenic material in amniotic fluids are more complete, and demonstrated that "heavy smokers may expose their unborn children to mutagenic substances" (22). The studies using the  $^{32}\text{P}$ -postlabeling assay demonstrated a clear association between cigarette smoking and *in vivo* formation of covalent DNA adducts in humans. Even relatively modest use of cigarettes induces modifications in genetic material in the placenta. The findings suggested that the major adducts being formed by cigarette smoking *in vivo* may not be caused by several of the most intensely studied polycyclic aromatic hydrocarbons or by aromatic amines in cigarette smoke. Data from this assay also suggested the presence of several adducts not associated with smoking; investigation of their origin will require analysis of additional specimens. This approach may ultimately lead to the definition of chemical components of cigarette smoke and other environmental exposures that most severely damage DNA *in vivo* (52,58,59,63). The approach may also be used to investigate the extent that adducts are formed in specific tissues. Likewise, if coupled with assays of the same study subjects for enzyme inducibility or DNA repair, this approach may be useful for studying associations between metabolic or repair capacities of tissues or individuals and adduct levels. (In fact, it is possible that some of the smoking-related adducts are a result of smoking-associated induction of enzymes that cause endogenous chemicals to form adducts or loss of repair leading to persistence of adducts from other sources.)

Assays for sister chromatid exchanges in cord blood lymphocytes are the only example where a fairly comprehensive series of studies did not demonstrate effects from smoking. The absence of a smoking effect on the frequency of SCEs in cord blood may be because of lack of transplacental passage of substances responsible for this effect, heightened DNA repair processes in the fetus, an inadequate period of exposure of the newly arising lymphocytes in the fetus, or other causes (34).

### Implications for Studies of Determinants of Genetic Damage Other Than Smoking

The results of these investigations suggest several principles for studies of other assays and agents. First, investigations of smoking demonstrate that there are major advantages to using situations in which exposure is relatively well defined for initial evaluations of the usefulness of different assays for genetic damage. Stud-

ies of smoking are frequently the best available model, and, given the number of potential study subjects who are exposed to tobacco smoke, the effects of smoking will often have to be understood in order to investigate effects of other agents. Second, characterization of the exposures by multiple questionnaire and laboratory approaches can enhance understanding gained in such studies, as illustrated by the clearer demonstration of a dose-response relationship between the intensity of smoking exposure and levels of adduct 1 when this association was sought using the biochemical data rather than the questionnaire data. Third, while most of the studies reviewed considered only a single assay, it seems likely that evaluation of assays would be facilitated by including multiple end points such as chromosomal damage and gene mutation in the same study whenever possible. Finally, studies of smoking typically point to the most useful available assays for assessing effects of other environmental exposures and provide a means for judging desirable qualities of assays and prioritizing assays for further development. For example, in addition to its great sensitivity, the  $^{32}\text{P}$ -postlabeling assay has advantages of great specificity, allowing it to contribute to identification of critical components in complex mixtures and to provide leads to unrecognized hazards. Likewise, the technique for assaying for 6-thioguanine-resistant lymphocytes that provides clones of mutant cells provides not only frequencies of mutation, but also information to characterize the molecular nature of the mutational damage. Emphasis should be given to development of other assays that yield results that are highly specific as well as quantitative.

### Implications for Future Epidemiologic Studies

The availability of highly sensitive and specific measures of damage to genetic material should provide exciting tools for future epidemiologic studies investigating either prenatal or postnatal determinants of disease risk. Such assays may be both sensitive enough to investigate effects of ubiquitous low-level exposures and/or specific enough to be used to study small numbers of cases or clusters of disease. Ideally, results of individual assays would be linked with other assays to provide convincing data on the public health risk from an exposure. Table 3 presents a hypothetical example of such a study. Note that in this scenario a single assay is not used to connect exposure and disease, but different assays are used to provide evidence for exposure, biological effect, effect in target tissue, and health effects. Each of these multiple assays provides a slightly different piece of the biologic puzzle, that together may constitute a compelling case for a causal association. The future availability of these biomarkers should provide a means for investigating many areas that are difficult to approach using the tools currently available to environmental epidemiologists. One can conceive this approach as providing a means to study the etiology of

**Table 3. Data available for risk assessment from a hypothetical future molecular epidemiologic investigation.**


---

DNA adducts are found in human tissues.
Exposures of these individuals suggest a source of adducts.
Environmental samples produce the same adducts in animal or <i>in vitro</i> models.
Specific fractions of the environmental samples produce high levels of adducts, allowing their chemical characterization.
The adducts are at positions on nucleic acids prone to causing specific types of mutations.
Increased frequencies of similar mutations are observed in cells of exposed humans.
Similar mutations are observed in activated oncogenes found in cancers of individuals with high levels of the exposure under study.
Exposed individuals whose metabolism causes high levels of the adduct or who repair these adducts poorly are at high risk of the tumor.

---

diseases including cancer, birth defects, spontaneous abortion, or the effects of occupational exposures or exposures to toxic wastes or dump sites.

Although in principle each of the assays needed for the hypothetical future epidemiologic study described in Table 3 are available, in practice there is much work to be done before such unified studies can be undertaken. Preliminary to such work, disciplinary and institutional barriers to progress in the evaluation and use of biomarkers for direct measurement of genetic damage in humans need to be overcome. Design of the most effective studies using biomarkers may require us to put aside, at least for a time, our disciplinary focus and approach these problems from a broader perspective. That perspective would draw on the most appropriate tools for the problem at hand from the disciplines of clinical research, epidemiology, toxicology, and molecular biology. (Perhaps this could be called the perspective of an environmental etiologist.)

Institutional barriers are a result of the fact that interest in and support for investigation of each of the factors that may cause genetic damage (genetic, life-style, dietary, occupational, pollutants, and other environmental factors) largely come from separate institutions. Likewise, the mandate for studying each of the diseases that may result from genetic damage (genetic diseases, cancer, adverse reproductive outcomes, aging, and others) largely resides in different institutions. This makes it difficult to achieve an overall perspective of the causes of genetic damage and tends to fragment efforts. Ways to coordinate efforts while using the strengths of these institutions need to be identified and encouraged.

Finally, each of the assays that would be used for our hypothetical future epidemiologic study needs to be further characterized and critically evaluated for its sensitivity, specificity, and overlap with other assays. This evaluation process should be done in an orderly manner, starting with use of the assays in model studies detecting effects of precisely characterized, high levels of exposure (such as patients receiving cancer chemotherapy or smokers). In these initial studies the study sub-

jects are to a large extent used to investigate properties of the assay. Understanding gathered in these model studies can then be used to develop studies where the assay is used to characterize determinants of disease in the population studied. When the molecular approach to epidemiology is as fully developed as are traditional approaches to epidemiology and toxicology, studies such as that described in Table 3 should help identify prenatal exposures causing the greatest risk to public health.

Assays for DNA adducts described here were conducted by Kurt and Erika Randerath and Tommie A. Avitts using support from USPHS grants CA 32157 and CA 43263, ES PR-547489, and a DuPont Occupational and Environmental Health Grant.

## REFERENCES

- Herbst, A. L., Ulfelder, H., and Poskanzer, D. C. Adenocarcinoma of the vagina: association of maternal stilbestrol therapy with tumor appearance in young women. *N. Engl. J. Med.* 284: 878-881 (1971).
- Greenwald, P., Barlow, J. J., Nasca, P. C., and Burnett, W. S. Vaginal cancer after maternal treatment with synthetic estrogens. *N. Engl. J. Med.* 285: 390-392 (1971).
- Herbst, A. L., Cole, P., Colton, T., Robboy, S. J., and Scully, R. E. Age-incidence and risk of diethylstilbestrol-related clear cell adenocarcinoma of the vagina and cervix. *Am. J. Obstet. Gynecol.* 128: 43-50 (1977).
- Everson, R. B. Individuals transplacentally exposed to maternal smoking may be at increased cancer risk in adult life. *Lancet* ii: 123-127 (1980).
- Preston-Martin, S., Yu, M. C., Benton, B., and Henderson, B. E. N-Nitroso compounds and childhood brain tumors: a case-control study. *Cancer Res.* 42: 5240-5245 (1982).
- van Steensel-Moll, H. A., Valkenburg, H. A., and van Zanen, G. E. Childhood leukemia and parental occupation. A register-based case-control study. *Am. J. Epidemiol.* 121: 216-224 (1985).
- Tomatis, L., Turusov, V., and Guibbert, D. Prenatal exposure to chemical carcinogens. In: *Topics in Chemical Carcinogenesis* (W. Nakahara, S. Takayama, T. Sugimura, and S. Odashima, Eds.), University Park Press, Baltimore, MD, 1972, pp. 445-459.
- Perinatal Carcinogenesis. NCI Monograph 51. NIH Publication No. 80-1633. National Cancer Institute, Bethesda, MD, 1979.
- Rice, J. M. Exposure to chemical carcinogens during pregnancy: consequences for mother and conceptus. *Adv. Exp. Med. Biol.* 176: 13-49 (1984).
- Anderson, L. M., Donovan, P. J., and Rice, J. M. Risk assessment for transplacental carcinogens. In: *Toxicity Testing: New Approaches in Toxicity Testing and Their Applications in Human Risk Assessment* (A. P. Li, Ed.), Raven Press, New York, 1985, pp. 179-202.
- U.S. Department of Health and Human Services, Public Health Service. *The Health Consequences of Smoking for Women: A Report of the Surgeon General*. Washington, DC, U.S. Government Printing Office, 1980.
- Sandler, D. P., Everson, R. B., Wilcox, A. J., and Browder, J. P. Cancer risk in adulthood from early life exposure to parents' smoking. *Am. J. Public Health* 75: 487-492 (1985).
- Sandler, D. P., Wilcox, A. J., and Everson, R. B. Cumulative effects of lifetime passive smoking on cancer risk. *Lancet* i: 312-315 (1985).
- Sandler, D. P., and Shore, D. L. Quality of data on parents' smoking and drinking provided by adult offspring. *Am. J. Epidemiol.* 124: 768-778 (1986).
- Perera, F. P., and Weinstein, I. B. Molecular epidemiology and carcinogen-DNA adduct detection: new approaches to studies of human cancer causation. *J. Chronic Dis.* 35: 581-600 (1982).
- Henderson, L. Transplacental genotoxic agents: cytogenetic methods for their detection. In: *Chemical Mutagens: Principles and Methods for Their Detection*, Vol. 10 (F. J. de Serres, Ed.), Plenum Press, New York, 1986, pp. 327-355.



17. Yamasaki, E., and Ames, B. N. Concentration of mutagens from urine by adsorption with the nonpolar resin XAD-2: cigarette smokers have mutagenic urine. *Proc. Natl. Acad. Sci. (U.S.)* 74: 3555-3559 (1977).
18. Legator, M. S., Bueding, E., Batzinger, R., Connor, T. H., Eisenstadt, E., Farrow, M. G., Ficsor, G., Hsie, A., Seed, J., and Stafford, R. S. An evaluation of the host-mediated assay and body fluid analysis: a report of the U.S. Environmental Protection Agency Gene-Tox Program. *Mutat. Res.* 98: 319-374 (1982).
19. Everson, R. B. Detection of occupational and environmental exposures by bacterial mutagenesis assays of human body fluids. *J. Occup. Med.* 28: 647-655 (1986).
20. Everson, R. B., Milne, K. L., Warburton, D., McClamrock, H. D., and Buchanan, P. D. Mutagenesis assays of human amniotic fluid. *Environ. Mutagen.* 7: 171-184 (1985).
21. Rivrud, G. N., Berg, K., Anderson, D., Blowers, S., and Bjoro, K. Study of the amniotic fluid from smokers and nonsmokers in the Ames test. *Mutat. Res.* 169: 11-16 (1986).
22. Rivrud, G. N., Berg, K., Anderson, D., Blowers, S., and Bjoro, K. Mutagenic effect of amniotic fluid from smoking women at term. *Mutat. Res.* 171: 71-77 (1986).
23. Kado, N. Y., Langley, D., and Eisenstadt, E. A simple modification of the Salmonella liquid-incubation assay. Increased sensitivity for detecting mutagens in human urine. *Mutat. Res.* 121: 25-32 (1983).
24. Obe, G., Heller, W. D., and Vogt, H. J. Mutagenic activity of cigarette smoke. In: *Mutations in Man* (G. Obe, Ed.), Springer-Verlag, Berlin, 1984, pp. 223-246.
25. Sorsa, M. Monitoring of sister chromatid exchange and micronuclei as biological endpoints. In: *Monitoring Human Exposure to Carcinogenic and Mutagenic Agents* (A. Berlin, M. Draper, K. Hemminki, and H. Vainio, Eds.), IARC Scientific Publications No. 59, Lyon, 1984, pp. 339-349.
26. Watanabe, T., and Endo, A. The SCE test as a tool for cytogenetic monitoring of human exposure to occupational and environmental mutagens. In: *Sister Chromatid Exchanges, 25 Years of Experimental Research, Part B, Genetic Toxicology and Human Studies* (R. R. Tice and A. Hollaender, Eds.), Plenum Press, New York, 1984, pp. 939-955.
27. Morimoto, K., Miura, K., Kaneko, T., Iijima, K., Sato, M., and Koizumi, A. Human health situation and chromosome alterations: sister chromatid exchange frequency in lymphocytes from passive smokers and patients with hereditary diseases. In: *Sister Chromatid Exchanges, 25 Years of Experimental Research, Part B, Genetic Toxicology and Human Studies* (R. R. Tice and A. Hollaender, Eds.), Plenum Press, New York, 1984, pp. 801-811.
28. Hansteen, I. L. Chromosome breaks in newborns exposed to tobacco smoke in utero. *Mutat. Res.* 130: 239 (1984).
29. Hatcher, N. H., and Hook, E. B. Somatic chromosome breakage in low birth weight newborns. *Mutat. Res.* 83: 291-299 (1981).
30. Aghamohammadi, S. Z., Henderson, L., and Cole, R. J. The human lymphocyte micronucleus assay. Response of cord blood lymphocytes to gamma-irradiation and bleomycin. *Mutat. Res.* 130: 395-401 (1984).
31. Ardito, G., Lamberti, L., Ansaldi, E., and Ponzetto, P. Sister-chromatid exchanges in cigarette-smoking human females and their newborns. *Mutat. Res.* 78: 209-212 (1980).
32. Seshadri, R., Baker, E., and Sutherland, G. R. Sister-chromatid exchange (SCE) analysis in mothers exposed to DNA-damaging agents and their newborn infants. *Mutat. Res.* 97: 139-146 (1982).
33. Lundberg, M. S., and Livingston, G. K. Sister-chromatid exchange frequency in lymphocytes of smoking and nonsmoking mothers and their newborn infants. *Mutat. Res.* 121: 241-246 (1983).
34. Lundgren, K., Lambert, J. M., Schreinemachers, D., and Everson, R. B. Effects of 5-bromo-2-deoxyuridine concentration and alpha-naphthoflavone on the association between smoking and the frequency of sister chromatid exchanges in lymphocytes from maternal and cord blood. *Mutat. Res.*, in press (1987).
35. Hill, A., and Wolff, S. Increased induction of sister chromatid exchange by diethylstilbestrol in lymphocytes from pregnant and premenopausal women. *Cancer Res.* 42: 893-896 (1982).
36. Sharma, T., and Das, B. C. Higher incidence of spontaneous sister-chromatid exchanges (SCEs) and X-ray-induced chromosome aberrations in peripheral blood lymphocytes during pregnancy. *Mutat. Res.* 174: 27-33 (1986).
37. Hatcher, N. H., and Hook, E. B. Sister chromatid exchange in newborns. *Hum. Genet.* 59: 389-391 (1981).
38. Das, B. C., Rani, R., Mitra, A. B., and Luthra, U. K. Baseline frequency of sister-chromatid exchanges (SCE) in newborn lymphocytes and its relationship to in vivo aging in humans. *Mutat. Res.* 144: 85-88 (1985).
39. Strauss, G. H., and Albertini, R. J. Enumeration of 6-thioguanine-resistant peripheral blood lymphocytes in man as a potential test for somatic cell mutations arising in vivo. *Mutat. Res.* 61: 353-379 (1979).
40. Albertini, R. J., Castle, K. L., and Borcharding, W. R. T-cell cloning to detect the mutant 6-thioguanine-resistant lymphocytes present in human peripheral blood. *Proc. Natl. Acad. Sci. (U.S.)* 79: 6617-6621 (1982).
41. Morley, A. A., Trainor, K. J., Seshadri, R. S., and Ryall, R. G. Measurement of in vivo mutations in human lymphocytes. *Nature* 302: 155-156 (1983).
42. Albertini, R. J. Somatic gene mutations in vivo as indicated by the 6-thioguanine-resistant T-lymphocytes in human blood. *Mutat. Res.* 150: 411-422 (1985).
43. Henderson, L., Cole, H., Cole, J., James, S. E., and Green, M. Detection of somatic mutations in man: evaluation of the microtitre cloning assay for T-lymphocytes. *Mutagenesis* 1: 195-200 (1986).
44. Turner, D. R., Morley, A. A., Haliandros, M., Kutlaca, R., and Sanderson, B. J. In vivo somatic mutations in human lymphocytes frequently result from major gene alterations. *Nature* 315: 343-345 (1985).
45. Albertini, R. J., O'Neill, J. P., Nicklas, J. A., Heintz, N. H., and Kelleher, P. C. Alterations of the hprt gene in human in vivo-derived 6-thioguanine-resistant T lymphocytes. *Nature* 316: 369-371 (1985).
46. Langlois, R. G., Bigbee, W. L., and Jensen, R. H. Flow cytometric characterization of normal and variant cells with monoclonal antibodies specific for glycophorin A. *J. Immunol.* 134: 4009-4017 (1985).
47. Jensen, R. H., Bigbee, W., and Branscomb, E. W. Somatic mutations detected by immunofluorescence and flow cytometry. In: *Biological Dosimetry, Cytometric Approaches to Mammalian Systems* (W. G. Eisert and M. L. Mendelsohn, Eds.), Springer-Verlag, Berlin, 1984, pp. 161-170.
48. Albertini, R. J., Dannenberg, B. D., Sylwester, D. L., Pettingill, C. A., and Kelleher, P. C. Thioquantine resistant lymphocytes in humans: placental blood variant frequencies are associated with exposures to potential mutagens. *Environ. Mutagen.* 5: 430 (1983).
49. Poirier, M. C. The use of carcinogen-DNA adduct antisera for quantitation and localization of genomic damage in animal models and the human population. *Environ. Mutagen.* 6: 879-887 (1984).
50. Strickland, P. T., and Boyle, J. M. Immunoassay of carcinogen-modified DNA. *Prog. Nucleic Acid Res. Mol. B* 31: 1-57 (1984).
51. Santella, R. M., Lin, C. D., Cleveland, W. L., and Weinstein, I. B. Monoclonal antibodies to DNA modified by a benzo[a]pyrene diol epoxide. *Carcinogenesis* 5: 373-377 (1984).
52. Everson, R. B., Randerath, E., Santella, R. M., Cefalo, R. C., Avitts, T. A., and Randerath, K. Detection of smoking-related covalent DNA adducts in human placenta. *Science* 231: 54-57 (1986).
53. Randerath, K., Reddy, M. V., and Gupta, R. C. <sup>32</sup>P-Labeling test for DNA damage. *Proc. Natl. Acad. Sci. (U.S.)* 78: 6126-6129 (1981).
54. Randerath, E., Agrawal, H. P., Weaver, J. A., Bordelon, C. B., and Randerath, K. <sup>32</sup>P-Postlabeling analysis of DNA adducts persisting for up to 42 weeks in the skin, epidermis and dermis of mice treated topically with 7,12-dimethylbenzo(a)anthracene. *Carcinogenesis* 6: 1117-1126 (1985).
55. Lu, L. J. W., Disher, R. M., Reddy, M. V., and Randerath, K. <sup>32</sup>P-Postlabeling assay in mice of transplacental DNA damage induced by the environmental carcinogens safrole, 4-aminobiphenyl, and benzo(a)pyrene. *Cancer Res.* 46: 3046-3054.



56. Liehr, J. G., Randerath, K., and Randerath, E. Target organ-specific covalent DNA damage preceding diethylstilbestrol-induced carcinogenesis. *Carcinogenesis* 6: 1067-1069 (1985).
57. Liehr, J. G., Avitts, T. A., Randerath, K., and Randerath, E. Estrogen-induced endogenous DNA adduction: possible mechanism of hormonal cancer. *Proc. Natl. Acad. Sci. (U.S.)* 83: 5301-5305 (1986).
58. Everson, R. B., Randerath, E., Avitts, T. A., Schut, H. A. J., and Randerath, K. Preliminary investigations of tissue specificity, species specificity, and strategies for identifying chemicals causing DNA adducts in human placenta. *Prog. Exp. Tumor Res.* 31: 86-103 (1987).
59. Randerath, K., Reddy, M. V., Avitts, T. A., Miller, R. H., Everson, R. B., and Randerath, E. <sup>32</sup>P-Postlabeling test for smoking-related DNA adducts in animal and human tissues. In: *Banbury Report 23: Mechanisms in Tobacco Carcinogenesis*, Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1986, pp. 85-98.
60. Simpson, W. J. A preliminary report on cigarette smoking and the incidence of prematurity. *Am. J. Obstet. Gynecol.* 73: 808-815 (1957).
61. Stein, Z., and Kline, J. Smoking, alcohol and reproduction. *Am. J. Public Health* 73: 1154-1156 (1983).
62. Everson, R. B., Randerath, E., Haley, N. J., Avitts, T. A., and Randerath, K. Quantitative associations between smoking and DNA adducts in human placenta measured by <sup>32</sup>P-postlabeling. *Proc. Am. Assoc. Cancer Res.* 28: 253 (1987).
63. Randerath, E., Avitts, T. A., Reddy, M. V., Miller, R. H., Everson, R. B., and Randerath, K. Comparative <sup>32</sup>P-analysis of cigarette smoke induced DNA damage in human tissues and mouse skin. *Cancer Res.* 46: 5869-5877 (1986).